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Review: Potential Antioxidants from Tropical Plants

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1. Introduction

1.1 Background

Higher plants have been utilised as important sources of medicinal drugs and health products since ancient days. Our ancestors practice the use of plants in their daily life as medicines. Therefore, the inherited knowledge about the traditional medicine is a key factor that could promote the development of modern drugs. Advances in modernisation and progress are expected to maximise the benefits of traditional herbal medicines for public health care (Chan, 2003). Investigations about plants have yielded amazing discoveries and development in modern medicine. Scientists carry out chemical investigation and purification of plants to get purified compounds which contribute to its medicinal properties.

The Malaysian rainforest is rich in diverse species of flora and fauna. Our forests store plenty of plant species which are important sources of traditional medicine. From about 10,000 species of higher plants and 2000 species of lower plants available in Peninsular Malaysia, approximately 16% are identified to be useful in medicines (Lattif et al., 1984). There are still a great number of unexplored plants that have high potential to be developed into medicines.

To date, a huge number of plants have been studied for their potential sources of antioxidants. Plants contain a wide variety of free radical scavenging molecules, such as polyphenols, dietary glutathione, vitamins and endogenous metabolites. These natural products make good antioxidants. Plant-derived antioxidants have been shown to function as singlet and triplet oxygen quenchers, peroxide decomposers, enzyme inhibitors and synergists (Larson, 1988). Studies have proven the correlation between the intake of fruits and vegetables and the morbidity and mortality from degenerative diseases (Rimm et al., 1996). It is not known which specific dietary constituents are responsible for this association, but antioxidants are assumed to be the major compounds that play an important role (Gey et al., 1991). Epidemiological studies that analyse the health implications of dietary components rely on the intake estimates in sample populations found in databases that list the component's content in commonly consumed foods (Pellegrini et al., 2003).

Besides dietary sources, antioxidants can be obtained in bulk from food processing industries and agricultural-by-products. The respective by-products are seeds, peels, bark, mill wastes and trimming wastes. In the citrus industries, industrial-by-products may

account for up to 50% of the total fruit weight (Bocco et al., 1998). The utilisation of the by-products in those industries is beneficial to both the economy and the environment. The industrial-by-products, like peel and seed, are proven to have high antioxidant level which is even higher than the flesh and other parts of the fruit with the presence of high polyphenol content. This is true for *Viburnum opulus* seed (Cam et al., 2007), peach peel (Chang et al., 2000), apple peel (He & Liu, 2007), mangosteen peel (Moongkarndi et al., 2004) and grape seed and skin (Rockenbach et al., 2011).

Numerous studies have been carried out on other potential agricultural-by-products such as trees. They serve as one of the cheapest available source of antioxidants. Among different parts of the plants, leaves receive special attention, e.g. Etlingera genus (Chan et al., 2011), Olea europaea (Silva et al., 2006), *Ligustrum vulgare* (Agati et al., 2009) and *Stevia rebaudiana* (Tadhani et al., 2007) ; bark from *Casuarina equisetifolia* (Zhang et al., 2010), *Acacia confusa* (Chang et al., 2001), *Populus tremuloides Michx* (Diouf et al., 2009); root of *Medicago sativa* (Dalton et al., 1998) and *Carissa spinarum* (Hegde & Joshi, 2010) were also reported to contain antioxidants.

Despite the utilisation of agricultural-by-products for human consumption, the safeties of the products have raised much attention. Dietary exposure to heavy metals, especially cadmium (Cd), lead (Pb), zinc (Zn) and copper (Cu), has been identified as a risk to human health. Heavy metals may be present in trace amounts occurring naturally in plants grown in the soil (Boruvka et al., 1997). Heavy metals have also been found in herbal medicines from Malaysia, e.g. *Eurycoma longifolia* products (Ang et al., 2003). Studies in Malaysia showed that only 92% of the products complied with the quality requirement for traditional medicines in the country, however, they cannot be assumed to be safe from lead contamination because of batch-to-batch inconsistency (Ang et al., 2003). Cadmium is reported to accumulate in the kidney. There is overwhelming evidence that the cadmium induced tubular damage which is irreversible (Järup et al., 1998). Therefore, it is important to ensure that plants that are consumed by humans do not contain heavy metals higher than the permissible levels. This has to be monitored carefully to ensure the safety of plant parts used as nutraceuticals.

Generally, antioxidants extracted from plants show prooxidant activity at low concentration and antioxidant activity at higher concentrations (Yen et al., 1997). However, the opposite effect was observed in the case of ascorbic acid in the presence of transition irons (Halliwell, 1996). These findings remind us the importance of quantifying the prooxidant capacity of an extract that exerts high antioxidant activity and to interpret net antioxidant potential.

To date, there is no information concerning the profile of Malaysian plants, its antioxidant/prooxidant activity, cytotoxicity, heavy metal contamination and method of standardisation. The purpose of this study is to bridge this gap of knowledge. Direct beneficiaries of this research would be the general public, the herbal industries and the natural product researchers in Malaysia and elsewhere.

2. Assessment of antioxidant capacity and cytotoxicity of selected malaysian plants

Malaysia is rich in its biodiversity with over 12,000 flowering plant species, many of which are currently being used in traditional medicine. To date, a huge number of plants have been studied for its potential source of antioxidants. However, there exist no reports on the antioxidant activity, heavy metal and elemental analysis and cytotoxicity of Malaysian

plants which are potential sources of new antioxidants. A study was carried out in our laboratory to evaluate selected Malaysian plants for its free radical scavenging, inhibition in lipid peroxidation, phenolic and content. This was followed with the heavy metal and elements analysis and its cytotoxicity against several cell lines. The plants were chosen based on its use in traditional application within the region. The investigation is essential to establish the phenolic content of selected Malaysian plants, its capability as potential antioxidant and ensure its safety (Ling et al., 2010a). Figure 1 shows the cytotoxicity activity of the selected Malaysian plants.

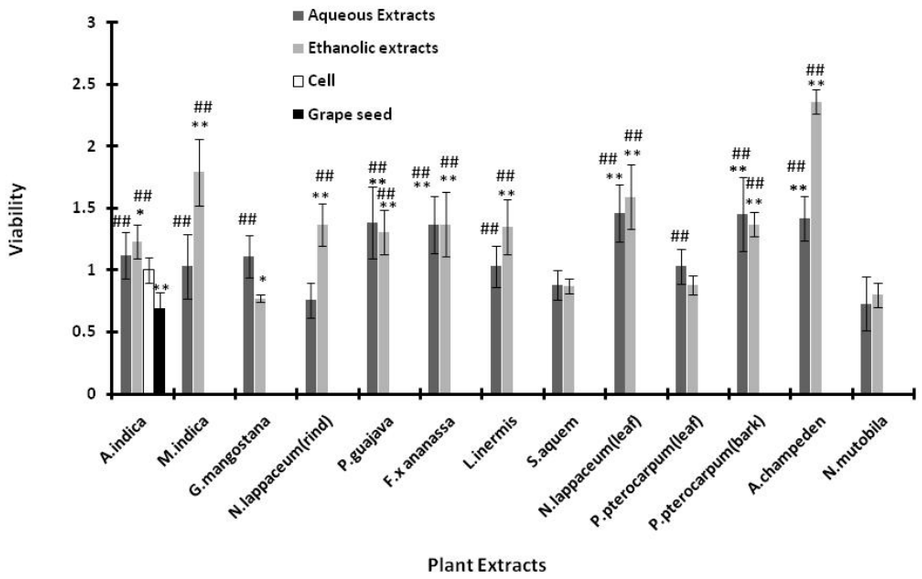

Fig. 1. Cytotoxicity activity of selected Malaysian plants on 3T3 mouse fibroblast cell at the concentration of 100µg/mL. * designates significant difference from cell alone ($P< 0.05$), ** designates a significant difference from cell alone, ($P< 0.01$), ## designates a significant difference from grape seed ($P<0.01$).

In our study, ethanolic and aqueous extracts of more than seventy Malaysian plants were screened using the DPPH assay. After the initial screen, thirteen plant extracts were selected with IC_{50} values lower than 5mg/mL and further tested with other free radical scavenging assays and for its ability to inhibit lipid peroxidation.. The extracts were also evaluated for its total phenolic content, heavy metal content and cytotoxicity. In general, the ethanolic extracts were observed to be far better free radical scavengers than the aqueous extracts. Some of the extracts were more potent (IC_{50} values) than the commercial grape seed antioxidant preparation (Agricultural Research Institute Speyer, Germany) and vitamin C. Generally, ethanolic extracts showed better activity in free radical scavenging assays and inhibition in lipid peroxidation compared to their aqueous extracts as well as higher than the commercial grape seed preparation. Most of the selected plant extracts showed hardly any heavy metal contamination in the powderised plants, some extracts even showed the presence of essential trace mineral. Majority of the plant extracts did not exhibit anti-proliferative effects on cultured mouse fibroblast and breast cancer cell indicating that most of the plant extracts are not cytotoxic to the cell been studied. We also observed a positive correlation between the ethanolic extract, phenolic content and antioxidant activity. We can

conclude that although a broaden use of these plants are in aqueous form, its commercial preparation can be achieved using ethanol since a high total phenolic content and antioxidant activity was seen in this preparation. It is desirable that these extracts be further purified to gain a better understanding of the active compounds contributing to its antioxidant activity.

Similar results were observed in the lipid peroxidation inhibition studies (Palanisamy et al., 2008). There was a strong correlation between antioxidant activity and the total phenolic content of the extracts. The high antioxidant extracts had below the permissible value of heavy metal content for nutraceutical application. Most of the extracts were also not cytotoxic to 3T3 and 4T1 cells at concentration as high as 100µg/mL (Ling et al., 2010a).

3. Prooxidant/antioxidant ratio (proantidex) as a better index of net free radical scavenging potential

Antioxidants are substances that protect other chemicals of the body from damaging oxidation reactions by reacting with free radicals and other reactive oxygen species within the body, hence hindering the process of oxidation (Halliwell & Gutteridge, 1995). Plants contain active components namely phenolics and polyphenolics that are known to act as antioxidants (Cai et al., 2003).

Every antioxidant is in fact a redox agent and might become a pro-oxidant to accelerate lipopolysaccarides and induce DNA damage under special conditions and concentrations. Studies have revealed pro-oxidant effects of antioxidant vitamins and several classes of plant-derived polyphenols such as flavonoids (Rahman et al., 1990) and tannins (Singh et al., 2001). As reported earlier, resveratol (Lastra & Villegas, 2007), phloroglucinols from *Garcinia subelliptica* (Wu et al., 2008) and curcumin (Ahsan & Hadi, 1998) can exhibit pro-oxidant properties, leading to oxidative breakage of cellular DNA in the presence of transition metal ions such as copper. Therefore, it is essential to discover natural compounds that good antioxidant activity but low pro-oxidant capabilities.

Pro-oxidant and antioxidant effect of plant extracts are due to the balance of two activities, free radical-scavenging activity and reducing power on iron ions, which may drive the Fenton reaction via reduction of iron ions. In a Fenton reaction, Fe^{2+} reacts with H_2O_2, resulting in the production of hydroxyl radical, which is considered to be the most harmful radical to biomolecules. Fe^{2+} is oxidized to Fe^{3+} in the Fenton reaction initially. By the action of many reductants, such as ascorbic acid, the oxidized forms of iron ion can be reduced to reduced forms (Fe^{2+}) later, which can enhance the generation of hydroxyl radicals. A predominant reducing power (on iron ions) over the free radical-scavenging activity in a mixture of compounds results in the pro-oxidant effect (Tian & Hua, 2005). In this study, the pro-oxidant capacity of the extracts were compared to the IC_{50} (mg/mL) of the antioxidant scavenging activity of DPPH radical. This ratio of pro-oxidant/antioxidant activity enabled us to evaluate the net antioxidant capacity of the extracts as this index will include not only the effective free radical-scavenging ability, taking into account pro-oxidant effect of the extracts as shown in equation (1).

$$\text{ProAntidex}=\frac{\text{Prooxidant capacity at the absorbance set at arbitrary 1.0 } (\text{mg}/\text{mL})}{IC_{50}(\text{mg}/\text{mL}) \text{ from DPPH scavenging assay}} \quad (1)$$

Ethanolic Extract	Plant Part	DPPH (IC_{50}, mg/ml)	Pro-oxidant (mg/ml)	Pro-Antidex
Azadirachta indica	leaf	0.74±0.46	0.50±0.02	0.91±0.58
Mangifera indica	leaf	0.17±0.02	0.22±0.03	1.32±0.29
Garcinia mangostana	peel	0.11±0.02	0.17±0.04	1.62±0.40
Nephelium lappaceum	**peel**	**0.12±0.05**	**0.05±0.04**	**0.48±0.37**
Psidium guajava	leaf	0.18±0.08	0.20±0.03	1.27±0.60
Fragaria x ananassa	**leaf**	**1.87±0.80**	**0.99±0.34**	**0.64±0.45**
Lawsonia inermis	**leaf**	**1.3±0.18**	**0.54±0.07**	**0.43±0.11**
Syzygium aqueum	**leaf**	**0.22±0.02**	**0.13±0.03**	**0.62±0.13**
Nephelium lappaceum	leaf	0.33±0.03	0.76±0.42	2.37±1.40
Peltophorum pterocarpum	leaf	0.17±0.12	0.11±0.03	0.83±0.44
Peltophorum pterocarpum	bark	0.10±0.04	0.08±0.03	0.94±0.66
Artocarpus champeden	leaf	0.30±0.21	0.75±0.51	3.82±4.24
Nephelium mutobile	leaf	0.24±0.03	0.18±0.06	0.76±0.28
Vitis vinifera	**seed**	**0.15±0.10**	**0.07±0.02**	**0.59±0.33**

Table 1. DPPH scavenging activity, Pro-oxidant activity and ProAntidex in ethanolic extracts of selected Malaysian plants and standard. ProAntidex was devised using the ratio of pro-oxidant activities to the IC_{50} DPPH scavenging activity. All values represent means ± SD, n=3.

Aqueous Extract	Plant Part	DPPH (IC_{50}, mg/ml)	Pro-oxidant (mg/ml)	Pro-Antidex
Azadirachta indica	leaf	0.96±0.14	1.13±0.01	1.20±0.18
Mangifera indica	leaf	0.49±0.39	1.03±0.88	2.03±1.38
Garcinia mangostana	peel	1.66±2.4	3.04±0.08	7.26±6.12**
Nephelium lappaceum	peel	0.54±0.15	0.55±0.37	1.08±0.65
Psidium guajava	leaf	0.22±0.01	0.42±0.33	1.89±1.43
Fragaria x ananassa	leaf	0.37±0.07	0.58±0.003	1.60±0.29
Lawsonia inermis	**leaf**	**3.71±0.34**	**1.41±0.21**	**0.38±0.06**
Syzygium aqueum	leaf	0.33±0.07	0.26±0.09	0.88±0.51
Nephelium lappaceum	leaf	0.67±0.02	>2	NA
Peltophorum pterocarpum	leaf	0.16±0.05	0.17±0.01	1.14±0.45
Peltophorum pterocarpum	bark	0.20±0.12	0.23±0.01	1.48±0.88
Artocarpus champeden	leaf	0.22±0.01	0.20±0.001	0.93±0.05
Nephelium mutobile	**leaf**	**3.76±0.27**	**0.18±0.01**	**0.05±0.01**
Vitis vinifera	**seed**	**0.46±0.18**	**0.24±0.11**	**0.59±0.35**
Green tea	NA	0.28±0.04	0.23±0.07	0.82±0.28
Emblica™	NA	0.31±0.07	0.27±0.12	0.69±0.18
Vitamin C	NA	0.01±0.00	0.03±0.35	4.10±3.36

Table 2. DPPH scavenging activity, pro-oxidant and ProAntidex in aqueous extracts of selected Malaysian plants and standards. ProAntidex was devised using the ratio of pro-oxidant activities to the IC_{50} DPPH scavenging activity. All values represent means ± SD, n=3. **Designates a significance difference from Emblica™, p<0.01.

The plant extracts having a high antioxidant activity were simultaneously analyzed for its pro-oxidant capability. Interestingly, in our laboratory, we established a Pro-oxidant/Antioxidant ratio (ProAntidex) which represents an index of the net free radical scavenging ability of whole plant extracts. The ethanolic extracts, *Nephelium lappaceum* peel, *Fragaria x ananassa* leaf, *Lawsonia inermis* leaf, *Syzygium aqueum* leaf and grape seed had lower Pro-Antidex than the commercially available Emblica™ extract which is a commercially available extract from *Phyllantus emblica* claims to have high antioxidant but low pro-oxidant activity (Table 1) (Ling et al., 2010b).

Among the aqueous extracts on the other hand; *Lawsonia inermis* leaf, *Nephelium mutobile* leaf and grape seed had low pro-oxidant activity (Table 2). In this study, Emblica™, green tea, vitamin C and grape seed were used as the positive controls in comparison to other plant extracts as shown in Figure 2. Among these extracts, the aqueous extract of *Nephelium mutobile* leaf had a very low ProAntidex of 0.05 compared to 0.69 for Emblica™. Most of the extracts had a far lower ProAntidex value compared to vitamin C. This index enables us to identify extracts with high net free radical scavenging activity potential. The ProAntidex is therefore beneficial as a screening parameter that can be used in food and healthcare industries.

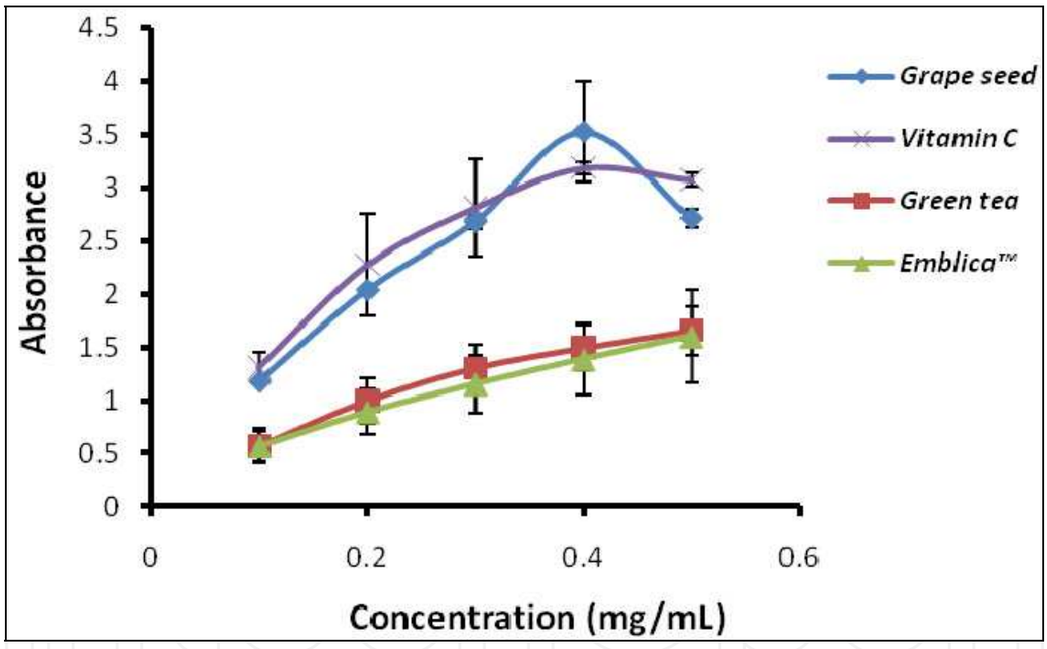

Fig. 2. Prooxidant activity of the standards used in the study. The prooxidant assay was carried out by measuring reducing power on Fe3+ in the Fenton reaction. Grape seed, green tea, Emblica ™ and vitamin C were used as positive controls. All the values represent means ± SD, *n*=3.

Pro-Antidex is a useful indicator in free radical research. The ratio of pro-oxidant to the antioxidant activity capacity gives a better picture of the real antioxidant capacity of the plant extracts. The pro-oxidant assay will enable the nutritionists and chemists to formulate antioxidant mixtures that balance between the two activities, which is higher antioxidant activity with lower pro-oxidant capacity. In other words, the net ProAntidex should be low to reflect that the particular plant extract has good overriding antioxidant property.

4. Standardised *Mangifera indica* leaf extract as an ideal antioxidant

Mangifera indica, commonly known as the mango plant has been the focus of many researchers for the next source of potent anti-oxidants. Previously, a standardised aqueous extract from the bark of *Mangifera indica* was reported to contain anti-inflammatory activity immunomodulatory and antioxidant activities (Garrido et al., 2004). The extract, is composed of a variety of phenolic acids, phenolic esters, flavanols and the xanthone mangiferin (Janet et al., 2006). When fed orally to mice that have been induced to have ear oedema by arachidonic acid and phorbol myristate acetate injection, the ear edema was observed to reduce markedly. *In vitro* studies showed that the extract also inhibited the induction of prostaglandin E (PG_E) and leukotriene-B_4 (LTB_4) release by macrophages (Garrido et al., 2004).

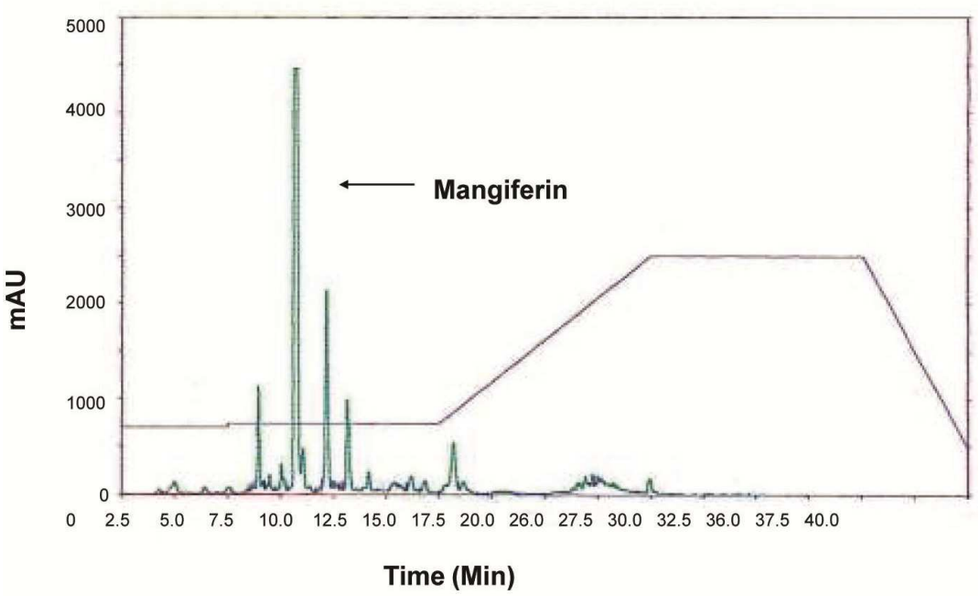

Fig. 3. HPLC chromatogram of *M. indica* extracts and standard, mangiferin. The mobile phase consisted of solvent A: 3% acetic acid in water and solvent B : acetonitrile; starting from 90%B for 5 minutes, 80%B for 15minutes and finally 100% B for 10minutes for washing and recondition the column (a) Mangiferin (b) ethanolic *M indica* extract and (c) aqueous *M indica* extract.

In our laboratory, the standardised ethanolic and aqueous extracts of Mangifera indica leaf was analyzed for its free radical scavenging activity using a variety of other assays. Its IC50 values using the DPPH assay was 0.17mg/mL ± 0.02 and 0.49mg/mL ± 0.4 respectively. Mangiferin, the main active compound in M.indica plant has been established to contribute to its biological activities (Ling et al., 2009). Standardised ethanolic extracts of the Mangifera indica leaf was found to have a mangiferin concentration of 71mg/g extract, free radical scavenging activity (IC50) of 0.17mg/mL ± 0.02 and total phenolic content of 590mg/g ± 48.08 of extract. The protection seen by Mangifera indica extracts against lipid peroxidation was observed to be far better than butlylated hydroxytoluene (BHT; a commercial anti oxidant used to prevent rancidity of oils) and commercial grape seed extract. The Mangifera indica extracts at higher concentrations did not exhibit pro-oxidant activities when compared to Vitamin C is yet another interesting feature of this extract. We also found that

the aqueous and ethanolic extracts of Mangifera indica leaf protects the mouse fibroblasts cells, NIH/3T3, from oxidant-induced cell death by about 84% and it is also non-toxic to cultured splenocytes .

5. Rind of rambutan, *Nephelium lappaceum*, a potential source of natural antioxidant

Nephelium lappaceum L. belongs to the same family (Sapindaceae) as the sub-tropical fruits lychee and longan and it is native to Southeast Asia. This fruit is an important commercial crop in Asia, where it is taken freshly or processed. In Southeast Asia, the dried fruit rind has been employed in traditional medicine for centuries. Additionally, the rind is used in cooking and the manufacture of soap. The roots, bark, and leaves have various uses in medicine and in the production of dyes. Previous studies have shown *N.lappaceum* rind extract to exhibit high antioxidant activity (Palanisamy et al., 2008), antibacterial activity (Thitilertdecha et al., 2010) and anti-Herpes Simplex virus type 1 (Nawawi A, 1999). Recently in our laboratory, *N.lappaceum* rind was also shown to have anti hyperglycemic potential (Palanisamy, Uma et al., 2011). The utilisation of *Nephelium lappaceum* rind to manage hyperglycemia is seen as an important finding not only in traditional medicine but also in aspects of valorisation of food waste.

The rind of *Nephelium lappaceum* (rambutan) was selected as the rind contains extremely high antioxidant activity when assessed using several free radical scavenging methods. Although having a yield of only 18%, the ethanolic rambutan rind extract has a total phenolic content of 762 ± 10 mg GAE/g extract, which is comparable to the commercial grape seed extract. The rambutan rind had lower pro-oxidant activities compared to vitamin C, α-tocopherol, grape seed and green tea in a dose response experiment. In addition, the rind extract at 100 µg/ml reduced oxidant-induced cell death (DPPH at 50 µM) by apoptosis to an extent similar to that of grape seed extract. The rind extracts were not cytotoxic to normal mouse fibroblast cells or splenocytes. Powderised rind had low heavy metal content far below the permissible levels for nutraceuticals. This study is the first to show a unique combination of high phenolic content, low pro-oxidant capacity and strong antioxidant activity of the rind extract of *Nephelium lappaceum*.

Whole extracts of the rind of *N.lappaceum* was standardized using a reverse phase column on analytical HPLC. Bioassay-guided fractionation of the extract was attempted to establish the most effective method to extract fractions with high antioxidant activity. Two fractionated extracts of the rind having DPPH activity of 0.01±0.001 and 0.01±0.003mg/ml and total phenolic content of 6662±240 and 1761±239 mg/g GAE respectively were established using preparative HPLC.

Bioassay guided fractionation was found to be time and labour consuming; therefore we investigated a rapid purification method to isolate and purify the bioactive compound from *N.lappaceum* rind extract. It was pertinent that we isolate and identify the active compound(s) in this extract that contribute(s) to the said biological activities. Structural characterization of purified compounds can lead to the formulation of the new therapeutic products. Geraniin was found to be the major phenolic compound in the *N.lappaceum* rind extract. A composition of 13% geraniin contributed to the high free radical scavenging activity in the extract. The compound exhibited radical scavenging activity of IC_{50}; 3.8 µg/mL (DPPH radical test), 1.7 µg/mL(ABTS radical test) and 1.7 µg/mL (Galvinoxyl radical test). The compound also displayed very low pro-oxidant capabilities.

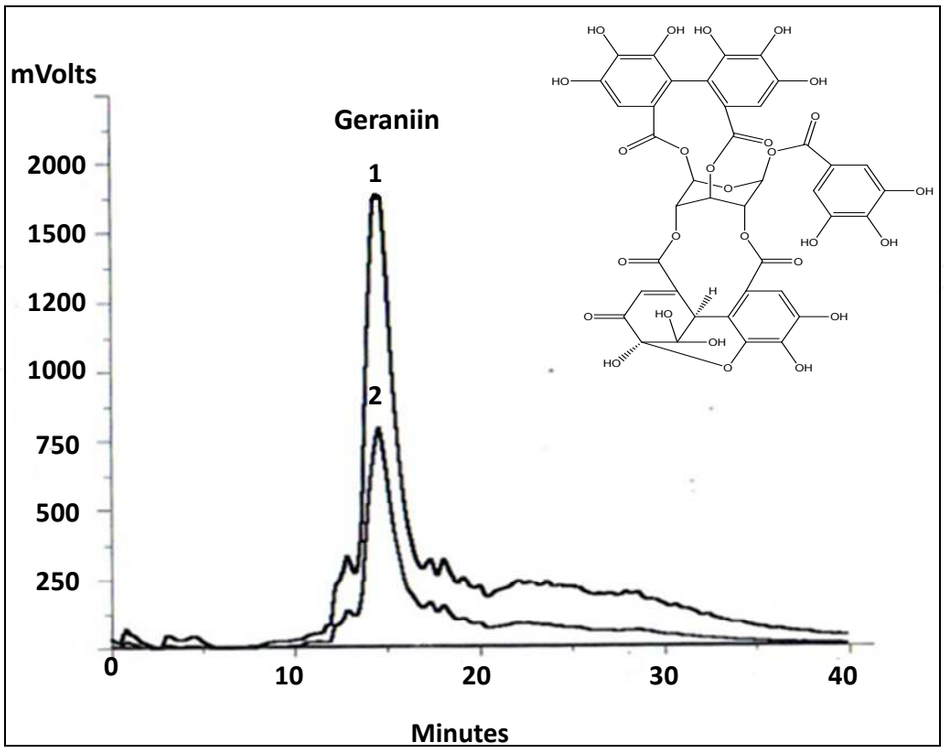

Fig. 4. Purification on Prep-HPLC showing geraniin as the major compound in the ethanolic *Nephelium lappaceum* rind extract. The solvent gradient consisted of 0-10% acetonitrile for 3 minutes, 10-40% for 12 minutes and finally 100% acetonitrile for 5 minutes to recondition the column. at a flow rate of 18mL/min. Geraniin was obtained at the retention time of 13minutes. 1 was detected at 210nm and 2 at 275nm. Insert shows the chemical structure of geraniin (Palanisamy, Uma D. et al., 2011).

Sample/Fraction	Extraction Method	Yield (%)	Geraniin (%) in sample
N.lappaceum rind	Ethanol extraction	30.58	3.79
Ethanolic extract	LiChroprep RP-18	60.00	12.68
F1	Preparative HPLC	21.15	21.13

Table 3. Quantification of geraniin in the rapid purification method

The rind of *N.lappaceum* extract was standardized to 13% of geraniin, the active hydrolysable ellagitannins responsible for over 50% of the antioxidant potential of the ethanolic extract of *Nephelium lappaceum* rind. In a single dose acute toxicity studies, oral LD_{50} of the rind extract in ICR mice was found to be greater than 5 g/kg body weight. In a subsequent study, Sprague Dawley rats were given via oral gavage 0 (control), 1000 mg/kg body weight/day of the extract for 28 days to evaluate the subacute toxicity of the extract to animals. Animals in a satellite group scheduled for follow-up observations were kept for 14 days without treatment to detect for any delayed effects. At the end of the experiment, kidney, liver, brain and testis were collected and followed by histopathological studies. No behavioural or organs to body weight changes were found in all the groups. Furthermore, no obvious abnormal changes were observed histologically in all the groups (unpublished data).

6. Conclusion

Malaysian plant extracts are a potential source of natural antioxidants. The chapter focuses on the identification of selected Malaysian plants that exhibit high antioxidant capability. We provide information concerning the complete profile of selected Malaysian plants on their antioxidant/pro-oxidant activity, cytotoxicity, heavy metal content and method of standardisation. In conclusion, it was established that *Nephelium lappaceum* rind and *Mangifera indica* leaf extract have great potential to be developed into an antioxidant nutraceutical. In future study, studies of membrane interaction and the regulation of antioxidant gene expression in the presence of extracts and their pure compounds in the cells will provide better understanding of the mode of actions of the antioxidant activity exhibited. *In vivo* subacute and chronic toxicity studies will need to be carried out to determine the effect of long term intake of *Nephelium lappaceum* rind extract and geraniin in the animals.

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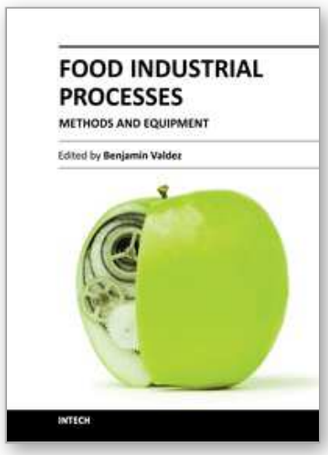

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The global food industry has the largest number of demanding and knowledgeable consumers: the world population of seven billion inhabitants, since every person eats! This population requires food products that fulfill the high quality standards established by the food industry organizations. Food shortages threaten human health and are aggravated by the disastrous, extreme climatic events such as floods, droughts, fires, storms connected to climate change, global warming and greenhouse gas emissions that modify the environment and, consequently, the production of foods in the agriculture and husbandry sectors. This collection of articles is a timely contribution to issues relating to the food industry. They were selected for use as a primer, an investigation guide and documentation based on modern, scientific and technical references. This volume is therefore appropriate for use by university researchers and practicing food developers and producers. The control of food processing and production is not only discussed in scientific terms; engineering, economic and financial aspects are also considered for the advantage of food industry managers.

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